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Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

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Abstract

Identification and quantification of Non Intentionally Added Substances (NIAS) at low concentrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As Raman spectroscopy techniques are innovative and sensitive analytical methods and do not require pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite (CaCO_3), calcium sulphate (CaSO_4), polystyrene (PS) and titanium dioxide (TiO_2) in anatase form. By a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO_3 , 1.62 mg/kg of CaSO_4 and 0.17 mg/kg of TiO_2 were estimated. The experimental findings achieved by Raman analysis were confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

Keywords	NIAS; Confocal Raman spectroscopy; Micro-Raman imaging; Food packaging;
Manuscript category	PLASTICS (chemical analysis including chemical analysis of Rubbers, polymers used in biomedical applications, scattering, spectroscopy, NMR)
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Data for: Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

Identification and quantification of Non Intentionally Added Substances (NIAS) at low concentrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As Raman spectroscopy techniques are innovative and sensitive analytical methods and do not require pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite (CaCO_3), calcium sulphate (CaSO_4), polystyrene (PS) and titanium dioxide (TiO_2) in anatase form. By a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO_3 , 1.62 mg/kg of CaSO_4 and 0.17 mg/kg of TiO_2 were estimated. The experimental findings achieved by Raman analysis were confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

April 29st, 2019

Dear Editor,

Please consider our manuscript for publication as an Article in “*Polymer Testing*” journal.

Manuscript Title: “*Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications*”

Category: *Article*

Authors: Chiara Portesi*, Davide Visentin, Francesca Dubiano, Maria Cesarina Abete, Marco Rizzi, Valter Maurino, and Andrea Mario Rossi

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In this work Raman spectroscopy has been applied for the first time for detecting and mapping with micrometric resolution the distribution of Non-Intentionally Added Substances (NIAS) present at the surface of Low Density Poly Ethylene (LDPE) pellets and extruded films intended for food packaging applications. Calcite (CaCO_3), calcium sulphate (CaSO_4), polystyrene (PS) and TiO_2 anatase were detected. For validating this method, the results of Raman spectroscopy analysis were compared to those obtained by ICP-MS and thus a semi-quantitative evaluation of the inorganic contaminants was possible, leading to the values of 0.167 mg/kg for TiO_2 and of 9.931 mg/kg CaCO_3 and 1.622 mg/kg of CaSO_4 respectively.

This work presents an original application of the Raman spectroscopy, suitable, due to its rapid and non-destructive character, for in-line measurements in food packaging industry.

The manuscript is submitted on an exclusive basis and its contents have not been published elsewhere.

Thank you for your consideration.

Sincerely,

Chiara Portesi

August 9th, 2019

Dear Editor, Dear Reviewers,

Thank you for considering for publication the manuscript "Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications".

The manuscript has been revised according to the comments of the reviewers.

Please find below the list of changes and comments

- The pellet provided by the buyer was guarantee as pure material, without any added substances. Applying Raman spectroscopy and ICP-MS we found presence of other elements, for this reason the term "NIAS" is used in the manuscript since pellets and films were provided to the buyer without any additive added in the samples in any stage of the production, except commercial antioxidants (as stated in rows 138-139). Therefore, all other material found can be considered as non-intentionally added.
- About polystyrene, due to the fact that it has been detected both on the surface of the pellets and in the bulk, its presence come from the production process of the granules, such as, for instance, from previous batch of other material (as stated in rows 281 – 282
- About TiO₂, even if the authors agree with the comment of the reviewer, several reports have confirmed that crystalline phases of TiO₂-NPs could play an important role in toxicity. However, there is still controversy over the degree or even the presence of effects due to the different surface properties and reactivity of the two allotropic phases of TiO₂-NPs, namely, anatase and rutile. Any case, the discussion in details of the toxicity of TiO₂ (or other NIAS) is beyond the purposes of this work. The aim of the manuscript is to propose Raman spectroscopy as simple, rapid, non-destructive and low cost for the detection on non-intentionally added substances in food packaging materials.
- The authors agree with the reviewers: the simple presence of a determined substance in FCM doesn't mean a risk. However, the proposed approach for the calculation of the transfer to food has the limit that the migration of the NIAS (as for any substance in contact with food) strongly depends on the type of food (foods with hydrophilic properties, such as aqueous, acidic and alcoholic foods, foods with lipophilic properties, such as dairy and non-dairy fatty foods, dry food, etc). Moreover, the migration tests are always performed in different conditions (temperature, time of storage, time of contact with the FCM). All this tests are beyond the purposes of this work.
- "Sensitivity" has been changed all along the manuscript with the more correct term "limit of detection", according with the reviewer comment.
- The references have been written in the adequate way.
- In rows 167-169, the choice of the calcination temperature of 500°C has been justified. In rows 169-170, the cleaning procedure of the crucible is described, performed in order to avoid any contamination of the samples. In rows 180.182, the choice of the glass crucible is justified.
- In row 228, the size of the sack is specified.
- Thank to the reviewer for the comments, in this work we decided to use the method of uncertainties type B. The 20% of uncertainty is a considerable value and it is the uncertainty of the technique (ICP-MS) for the given metals, in trace concentration.
- The determination of Al, Zn, Mg, Fe and Cu is done by ICP-MS but not confirmed by Raman spectroscopy – this is due to the fact that in the pellets they are present in a non-Raman active molecular (metallic) form. The measurements have been carried out in an analytical lab equipped for routine ICP-MS measurements avoiding any possible contamination during preparation and analysis process. The value of the blank is subtracted in the measurements.

- According to the reviewer comments, the captions have been condensed and table 1 removed.

Thank you for your consideration.

Sincerely,

Chiara Portesi

- Rapid and non-destructive identification of Non-Intentionally Added Substances by Raman spectroscopy;
- Determination of the aggregation state of the NIAS by Raman Imaging;
- Determination of the distribution of the NIAS in the LDPE matrix by Raman imaging;
- Semi-quantitative analysis of the NIAS;
- Comparison of Raman data and ICP-MS data

Development of a rapid micro-Raman spectroscopy approach for detection of NIAS in LDPE pellets and extruded films for food packaging applications

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ABSTRACT

Identification and quantification of Non Intentionally Added Substances (NIAS) at low concentrations and eventually their distribution on Low Density Polyethylene (LDPE) films is an important issue. As Raman spectroscopy techniques are innovative and sensitive analytical methods and do not require pretreatment and manipulation of the sample, these were used for the identification of NIAS in LDPE pellet and films, which are commonly used for food packaging applications. The aggregation state of NIAS in LDPE were observed by Raman imaging with micrometric resolution. Four main NIAS compounds were identified in granule and film surfaces by confocal Raman spectroscopy: calcite (CaCO_3), calcium sulphate (CaSO_4), polystyrene (PS) and titanium dioxide (TiO_2) in anatase form. By a semi-quantitative evaluation of the inorganic NIAS, 9.93 mg/kg of CaCO_3 , 1.62 mg/kg of CaSO_4 and 0.17 mg/kg of TiO_2 were estimated. The experimental findings achieved by Raman analysis were confirmed by the results obtained by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). Moreover, the toxicity of the identified compounds was examined according to the Cramer rules.

Keywords: NIAS; Confocal Raman spectroscopy; Micro-Raman imaging; Food packaging.

1. INTRODUCTION

In the last decades, concerns over food safety issues, such as possible contaminations due to migration of dangerous substances in food matrices from food packaging materials, have increased among consumers. Non Intentionally Added Substances (NIAS) in Food Contact Materials (FCMs) represent a major issue for the food packaging and FCM industry. In general, NIAS are chemical compounds that can be present in food packaging matrices and whose presence is unexpected and often undesired [1]. NIAS originate from break-down products of food contact materials, impurities of starting materials, unwanted side-products and various contaminants from recycling [2]. It is generally accepted that only compounds below 1000 Da are considered NIAS. Substances with a higher molecular weight are regarded as inert towards migration due to their larger sizes [3].

48 The safety of FCM must be evaluated on the basis of the amount of chemicals that can migrate from the
49 materials into food. The materials must be manufactured in compliance with EU regulations, including
50 good manufacturing practices, so that any potential transfer into food does not raise safety concerns.

51 The main risks concern the presence of unexpected and undesired chemicals which could be threaten
52 human health; other possible risks regard the change of the food composition with adverse effects on
53 the taste and odor. The Commission Regulation (EU) No. 10/2011 on plastic materials and articles
54 intended to come into contact with food states that potential health risks in the final food packaging
55 products or articles should be assessed by the manufacturer in accordance with the internationally
56 recognized principles of risk assessment. In that regard, the European Food Safety Authority (EFSA)
57 published a draft scientific opinion on recent developments in the risk assessment of chemicals in food
58 related to the safety assessment of substances used in FCM, for public consultation [4] in which
59 assessment of NIAS is briefly discussed. At the same time, the International Life Sciences Institute
60 (ILSI) published a guidance on best practices for NIAS evaluation in FCM [5,6]. Central to NIAS risk
61 assessment and the first step towards demonstrating NIAS compliance is to determine which substances
62 are present in the FCM. While NIAS derived from side reactions and degradation products can broadly
63 be predicted and used as a starting point for developing an analytical strategy, the NIAS due to
64 contamination can occur at any point in the life cycle of the FCM and their identification is considered
65 a significant challenge. Consequently, their individuation and quantification have to be carried out on
66 the finished materials and articles.

67 An effective approach to the NIAS testing and risk assessment solution is based on the following steps:
68 information gathering, analytical screening, identification of hazardous substances and final risk
69 assessment. Currently, screening programs to identify and semi-quantify the presence of NIAS are
70 executed with different sample preparation techniques (e.g. extraction with solvents under controlled

71 conditions) and analytical techniques (e.g. gas chromatography - quadrupole time-of-flight mass
72 spectrometry (GC/Q-TOF), gas chromatography - mass spectrometry (GC-MS), liquid chromatography
73 - mass spectroscopy (LC-MS) and inductively coupled plasma - mass spectrometry (ICP-MS)). By
74 using sensitive advanced analytical techniques most NIAS are regularly detected but not their
75 distribution. In the food packaging industry, the analytical techniques that are usually employed for
76 detecting NIAS rely on mass spectrometry, mainly GC-MS to quantify volatile [7–10] and semi-
77 volatile substance and triple quadrupole - Liquid Chromatography Mass Spectrometry (QqQ-LCMS) to
78 quantify non-volatile and polar compounds [11–13]. These are targeted analytical methods which need
79 internal standards composed of the same or similar chemical structures of the NIAS under
80 investigation. Moreover, for an accurate determination of the amount of NIAS present in the food
81 package, internal standards with also similar concentrations should be used.

82 Although GC-MS and LC-MS are powerful techniques, they have some limitations. In general, these
83 techniques are more suited for the evaluation of targeted chemical compounds or at least belonging to
84 the reference database for this reason unexpected chemical compounds could be difficult to identified.
85 Moreover, these two techniques have limitations with regard to the range of analytes that can be
86 handled. In addition, relatively to the ionization techniques compatible with to the LC-MS, MS
87 fragment determination is difficult when there is no *a priori* knowledge on the structure and origin
88 [1,3]. Lastly, all these determinations based on mass spectrometry are expensive and time-consuming,
89 thus their use in a lab as a possible in-line detection is infrequent. In addition, these techniques do not
90 provide any information about the distribution and the aggregation state of the contaminants in the
91 sample, which is an important parameter the packaging industry production process and for the risk
92 assessment purposes [4].

93 To overcome these issues, alternative techniques can be taken into account. Vibrational spectroscopies
94 have already proven to be powerful techniques for the identification of chemical compounds, thanks to
95 the unique chemicals spectra they provide. Moreover, these are able to perform spectra in few seconds,
96 and no pre-treatment of the sample is required. In particular, confocal Raman spectroscopy has the
97 additional advantages of not undergoing water interference and having a high spatial resolution, thus
98 allowing a chemical mapping of Raman active compounds on x-y plane with spatial resolution below 1
99 μm . In principle, confocal Raman spectroscopy enables an in-line characterization of granules and
100 films for what concern Raman active NIAS, since a single spectrum can be recorded in few seconds
101 and can provide information about their presence, their distribution and their aggregation state in the
102 FCM.

103 Thanks to these characteristics, Raman spectroscopy techniques have already been widely used in food
104 science for many years ([14–20] and they are promising candidate techniques for analysis in the food
105 packaging industry [21] to quickly detect the possible contamination of raw material and final products
106 by NIAS and their distribution in the FCM. Furthermore, by implementing these analytical methods,
107 i.e. using signal enhancement techniques such as Surface Enhanced Raman Spectroscopy (SERS) and
108 Tip-Enhanced Raman Spectroscopy (TERS), the limit of detection is further increased of orders of
109 magnitude and the resolution reaches the nanometer scale.

110 Nowadays, at industrial level the routine procedure for a preliminary screening of possible inorganic
111 impurities in granules and films intended for food packaging uses is the gravimetric ashes analysis [22].
112 However, this analysis has the main limitation of being a destructive technique, with a poor limit of
113 detection (limited to 0.01 mg for a commercial analytical balance) and no specificity. The operating
114 procedure consists in heating the organic matrix at high temperature (500 °C) so that this burns and, if

115 the resulting inorganic residues are significant by weight, these latter are subjected to further analysis
116 based on mass spectrometry.

117 In this work a versatile, simple and reproducible procedure to detect, discriminate and semi-quantify
118 NIAS in granules and extruded films of Low Density Polyethylene (LDPE) for food packaging
119 applications was proposed. The analytical procedure was set up on the raw material as a model system
120 to standardize the methodology for both qualitative and quantitative analyses. Moreover, micro-Raman
121 mapping was exploited to measure the distribution and the identification of contaminants on industrial
122 LDPE granule and films. The foreseen advantages rely on the rapidity and the low limit of detection of
123 the technique, on the possibility to use untreated raw materials, to not use internal standards and to map
124 the distribution of the contaminants and their aggregation state.

125 For a tentative semi-quantitative analysis of the inorganic NIAS, the ashes of the pellets were measured
126 by Raman spectroscopy. For a comparison, NIAS were determined also with established analytical
127 methods such as by gravimetric analysis and by ICP-MS. The toxicity of the identified compounds was
128 estimated on the basis of the Cramer classes.

129

130 **2. Materials and methods**

131 LDPE was chosen in this study as a representative of the common FCMs for food packaging
132 applications. In particular, the surface of 50 LDPE granules, their cross sections, and 10 portions of a
133 LDPE film were investigated for the possible presence of NIAS.

134 The LDPE samples were provided by Plastotecnica SpA. The film (width 100 cm and thickness 40 μm)
135 was obtained from the processing of the granules using a production blown line (die diameter 280 mm,
136 die gap 1.2 mm, mono-extrusion, line's speed 41 m/min). The density of LDPE was in the range of
137 0.921 g/cm^3 – 0.923 g/cm^3 and the melt flow index was in the range of 1.8 g/10 min – 2.0 g/10 min

(measured at 2.16 kg). The pellets were colorless and no additives were added in the samples in any stage of the production, except commercial antioxidants. Nitric acid (HNO₃ 68%) was purchased from Sigma-Aldrich (Milan, Italy) and was used for the preparation of the external standard solutions (1% in water) by dilution of a certified reference material and for the preparation of the solution samples to be injected in the ICP-MS. The solutions were prepared with Milli-Q quality water (18 MΩcm).

2.1 Qualitative analysis of LDPE granules and films

The LDPE granules and the extruded film were analyzed by Raman spectroscopy techniques for detection of possible NIAS both on the surface and in the bulk material. The samples were characterized without any preliminary treatment. In order to perform bulk material analyses, the granules were cut by a disposable scalpel and the internal surfaces were investigated by Raman spectroscopy. Confocal Raman spectra were obtained by a Thermo Scientific™ DXR™ Raman instrument. The microscope was equipped with a motorized stage with 1 μm of resolution in the X-Y plane and a charge-coupled device (CCD) detector. A preliminary optical investigation by using a 10× magnification objective of the surface of granules and film was performed for detecting possible inhomogeneities in the LDPE matrix. The spectra were then acquired in different points of the samples surface, by using a laser excitation source of 532 nm, 3 mW laser power and a 100× microscope objective, in the range from 50 cm⁻¹ to 3500 cm⁻¹ with a grating resolution of 5 cm⁻¹. In the case of detection of agglomerations which include contaminants, these latter were further characterized by micro-Raman imaging, which was carried out by a Thermo Scientific™ DXR^{xi} Imaging microscope, considering the same experimental parameters set for confocal spectra. This microscope is equipped with an Electron Multiplying CCD camera that allows to collect 600 spectra per second. In this way,

161 optical and chemical mapping of the contaminants was obtained, thus providing information of the
162 agglomeration state and dimensions of the NIAS with real-time data visualization.

163

164 **2.2 Ashes analysis of the LDPE granules**

165 For the ashes analysis, a 1.5 g of sample taken from LDPE granules were weighted on a glass crucible
166 and brought to a temperature of 500 °C. Then, the possible inorganic residues detected by gravimetry.
167 The calcination temperature of 500 °C has been chosen since all the organic components are thus
168 burned and removed, without any possible contamination from the crucible (the glass melting
169 temperature is 1723°C). As preliminary cleaning procedure, the crucible was soaked in aqua regia
170 (HCl:HNO₃ 3:1 v/v), rinsed thoroughly in water and dried with nitrogen. Then, it was degassed,
171 keeping it in the furnace at 105 °C for 2 h, and then it was weighted. These steps were repeated as
172 many time as needed for the crucible to be stable in weight.

173 An analytical balance SI 114 from Denver Instruments (maximum weight 110 g and resolution 0.1 mg)
174 was used to estimate the weight of the LDPE granules after thermal treatment. The thermal treatment
175 was carried out with a muffle Carbolite furnace RHF1400 and it consisted of a heating ramp from 20°C
176 to 500°C in 30 min and then in keeping the temperature at 500°C for 30 min. By this thermal treatment,
177 all the organic compounds of the samples burned and only inorganic residues, oxides and metals,
178 remained for the analysis.

179 After the thermal treatment, the inorganic residues possibly present in the crucible were detected by
180 optical inspection and analyzed by Raman spectroscopy and Raman imaging. The transparent glass
181 crucible allowed the back-illumination of the sample, thus helping in the detection even of the smallest
182 residual particles.

183 Then, ICP-MS technique was used for confirming by elemental analysis the presence of contaminants
184 detected by Raman characterization. A Thermo Scientific ICAP-Qs ICP-MS equipped with a
185 quadrupolar mass analyzer and a quadrupolar collision/reaction cell with flat electrodes (flatpole) was
186 used for the detection of Ca and Ti. This instrument was calibrated with the external standard solutions
187 composed of HNO₃ (1% in water) prepared by dilution of a certified reference material at 1 g/l, by
188 using ⁴⁵Sc (100 ppb) as internal standard. Interference from polyatomic ions were removed by
189 operating the collision cell He mode (the presence of He gas in the collision cell) with discrimination of
190 the ions kinetic energy (He – KED). The following ions were used for quantitation: ⁴⁴Ca, ⁴⁶Ti and ⁴⁷Ti.
191 Other operating parameter were: RF power 1450 W, Argon plasma gas flow 15 l/min, auxiliary gas
192 flow 1.0 l/min, nebulizer gas flow 0.90 l/min, sample flow rate 1.0 ml/min, nebulizer type concentric,
193 spray chamber impact bead, interface cones (sampler and skimmer) Ni collision cell, He flow 5.0
194 ml/min, extraction lenses potentials and KED bias optimized though the auto-tuning procedure by
195 using the tuning solution suggested by manufacturer.

196

197 **2.3 Evaluation of the toxicity of the identified NIAS**

198 Cramer classes were applied in order to estimate the toxicity of the identified compounds. Toxtree
199 v3.1.0 (Ideacon Ltd., Sofia, Bulgaria), based on a decision tree approach, was the used software.
200 The substances are classified into three classes taking into account their chemical structures: class I
201 corresponds to a low toxicity, class II corresponds to an intermediate toxicity and class III corresponds
202 to a high toxicity ([23]).

203

204 **3. Results and discussion**

205 After a preliminary investigation with an optical microscope of the whole surface of granules and film,
206 Raman spectra of the homogenous parts of LDPE samples were carried out (Fig. 1). The inset of Fig. 1
207 summarizes the assignment of the Raman bands to LDPE. The assignments are supported by Raman
208 spectra of several kinds of polyethylene obtained in previous studies [24]. The characteristic bands of
209 LDPE, clearly visible in Fig. 1, are: the symmetric CH₂ stretching mode at 2847 cm⁻¹, the asymmetric
210 CH₂ stretching mode at 2882 cm⁻¹, the unresolved feature near 1439 cm⁻¹ due to anisotropic parts (CH₂
211 Fermi resonance), CH₂ twisting features near 1295 cm⁻¹ and the amorphous C-C stretching at 1128 cm⁻¹
212 and 1062 cm⁻¹.

213 Thanks to the micro Raman spectroscopy was possible to observe inhomogeneous parts in LDPE
214 granules and films, composted of agglomerations with dimension of the order of μm². In fact, in the
215 optical image of Fig. 2 the agglomerations are visible in white, while the black background represents
216 the homogeneous parts of LDPE. The relatively large dimensions of these agglomerations are probably
217 due to the fact that contaminants tend to migrate within the polymer and to form aggregates. By
218 confocal Raman spectra four main compounds were identified as contaminants, as reported in Fig. 3
219 (a), (b), (c) and (d): calcite (CaCO₃), calcium sulphate (CaSO₄), polystyrene (PS) and titanium dioxide
220 (TiO₂) in anatase phase. Fig. 2 also shows the micro-Raman maps with CaCO₃, CaSO₄ and TiO₂
221 superimposed on the optical images. The combination of these images demonstrates the consistency of
222 the chemical and the morphological information. The same study was repeated for the bulk of the
223 granules. It was not possible to analyze inclusions of PS by micro-Raman imaging because in the time
224 needed to take the maps the laser degrades the organic materials, even if the laser power is reduced to
225 the minimum (1mW). The CaCO₃ was found on the surfaces both of the granules and film. Traces of
226 CaSO₄ were detected only on the surface of the granules. PS was found both on the surface and in the
227 bulk of the granules. TiO₂ anatase was found both on the surface of granules and film.

228 In order to establish if the possible source of contamination was the 25 kg sack containing the LDPE
229 pellets, a homogeneity study of the contaminants in granules accommodated in different location of a
230 sack was performed. Ten granules were allocated at different distance with respect to inner surface of
231 the sack and were analyzed in order to establish a possible contamination by the sack. Since no
232 differences were found in this sense, as an alternative it was assumed that the contamination originate
233 from the production process.

234 After the thermal treatment, micro-Raman imaging were used for the characterization of residues. An
235 area of about $1\text{ cm} \times 2\text{ cm}$ of the crucible containing the residues was characterized (See Fig. 4(a)). In
236 this area 22 aggregates were observed. Fig. 4(b) reports that 9 aggregates were composed of CaCO_3
237 (yellow squares), 2 of CaSO_4 (green squares), 8 of TiO_2 anatase (blue squares) and 3 of TiO_2 rutile
238 (black squares). The presence of TiO_2 rutile was due to the polymorph changes caused by the thermal
239 treatment, where a portion of TiO_2 anatase changed in rutile phase. Fig. 4 (c) and (d) show the residues
240 of CaCO_3 and of TiO_2 in anatase obtained by optical images and superimposed to the corresponding
241 micro-Raman images. No traces of PS were detectable after the thermal treatment.

242 Since these compounds, already present before the thermal treatment, were neither expected nor desired,
243 they can actually be considered as NIAS.

244 By gravimetric analysis, i.e. by weighting the crucible before and after the thermal treatment, no
245 residues were determined because the residues amount is below the resolution of the used analytical
246 balance (0.1 mg).

247 The analysis of residues of the LDPE granules by ICP-MS after the thermal treatment shows the
248 presence of Ca and Ti, with a measured concentration of 0.10 mg/kg and 3.585 mg/kg respectively,
249 with an instrumental uncertainty of about 20 % for both them. In this sense, the Raman and ICP-MS
250 analysis are consistent, since the results of Raman analysis show the presence of CaCO_3 , CaSO_4 and

251 TiO_2 in the LDPE matrices, and the ICP-MS data confirm the presence of Ca and Ti in the ashes
252 residues. Despite the concentration of these compounds is very low, at trace levels, the following
253 consideration can be done: i) assuming that all the Ti present in the LDPE samples is in form of titania
254 polymorph (TiO_2), a semi-quantitative estimation of the concentration of TiO_2 in the granules and film
255 can be carried out. Indeed, considering that the ratio between the atomic weights of O (15.999 u) and Ti
256 (47.867 u) in TiO_2 is 0.334 and that for each Ti atom two O atoms are present, from the results obtained
257 by ICP- MS that for Ti was of 0.1 mg/kg, a concentration of 0.17 mg/kg for TiO_2 was calculated. ii)
258 The large amount of Ca found in granules and film by elemental analysis is confirmed also by Raman
259 analysis by the presence of two compounds containing Ca (40.078 u): CaCO_3 and CaSO_4 . The
260 deductions on the respective concentration of the latter are more difficult than in case of TiO_2 . On the
261 other hand, by micro Raman imaging of the ashes on the crucible, a large abundance of CaCO_3 was
262 found compared to that of CaSO_4 (9 and 2, respectively, out of 22 aggregates in the investigated area).
263 Based on this consideration, a preliminary evaluation of the concentration of CaCO_3 and CaSO_4 was
264 done, with a similar consideration as above in the case of TiO_2 . If all the Ca present in the granules is in
265 form of either CaCO_3 (molecular weight 100 mg/mol) or CaSO_4 (molecular weight 136 mg/mol) in the
266 proportion of 9:2 then the concentration of CaCO_3 and CaSO_4 can be evaluated of 9.93 mg/kg and 1.62
267 mg/kg, respectively.

268 According to the Cramer classes, the inorganic NIAS, CaCO_3 , CaSO_4 and TiO_2 , detected by Raman
269 spectroscopy belong to the class III because they are not the normal constituents of a human body.
270 Moreover, NIAS contains substances other than C, H, O, N, divalent S, which don't curs only as a Na,
271 K, Ca, Mg, N salt, phosphate, sulphamate, sulphonate, sulphate and hydrochloride.

272 Concerning the traces detected by ICP-MS, any Raman active molecule was also detected by
273 spectroscopic analysis, therefore any further consideration can be done in this respect, except to the fact

the some NIAS are present in the samples, in the form of chemical compounds containing both transition metals and an alkaline earth metals (Mg). The largest abundance is measured for Al (1.19 mg/kg), Zn (0.67 mg/kg), Mg (0.56 mg/kg), Fe (0.45 mg/kg) and Cu (0.41 mg/kg).

In conclusion, Raman spectroscopy was demonstrated to be a sensitive technique for detecting NIAS in the LDPE granules and films intended for food packaging applications. The distribution of the NIAS between the surface and the cross-section of LPDE granules seem to indicate that CaCO_3 and CaSO_4 originate from the environmental contamination, since their presence was detected only at the surface of the granules, while the PS and TiO_2 contamination came from the production process of the granules, such as, for instance, from previous batch of other material, as they were found in bulk material as well.

By micro Raman imaging it is possible to understand the distribution of inclusions in the sample, and their agglomeration state. On this base it could be considered as a suitable technique for a semi-quantitative analysis of inorganic impurities in the surface of granules and films.

The limit of detection of Raman spectroscopy is below that of the gravimetric method, and it is able to discriminate the different types of contaminant. The results obtained by Raman spectroscopy were confirmed by the ICP-MS analyses.

This approach can be implemented for performing non-destructive and in-situ analysis of the surface of granules and films without any pre-treatment of the sample, in a little time-consuming and in very much effective way, thus addressing the stringent needs in food packaging industry related to food safety and risk assessment issues.

294

295 **Acknowledgment**

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297 (AIREf:27/97643).

298

299 **Data availability**

300 The raw data required to reproduce these findings cannot be shared at this time as the data also forms
301 part of an ongoing study. The processed data required to reproduce these findings cannot be shared at
302 this time as the data also forms part of an ongoing study.

303

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388 **FIGURE CAPTIONS**

389

390 Fig. 1 Raman spectra of the LDPE matrix and the assignments of Raman bands in the inset.

391

392 Fig. 2. Micro-Raman imaging of inhomogeneous parts of LDPE samples (white parts) inclosing the
393 agglomerations with CaCO₃ (a), CaSO₄ (b) and TiO₂ in anatase phase (c).

394

395 Fig. 3. Raman spectra of CaCO₃ (a), CaSO₄ (b), TiO₂ in anatase phase (c) and PS (d).

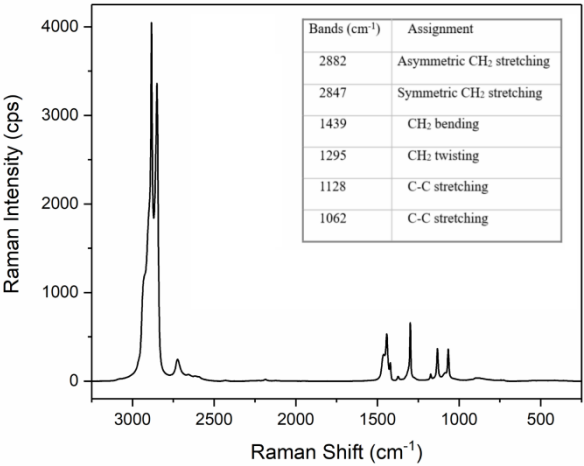
396

397 Fig. 4. Distribution of inorganic residues on the crucible (a) and their abundance (b). Optical images and micro-
398 Raman maps of CaCO₃ (c) and TiO₂ in anatase phase (d) residues in the crucible after thermal treatment.

399

400

401 Fig. 1



402

403

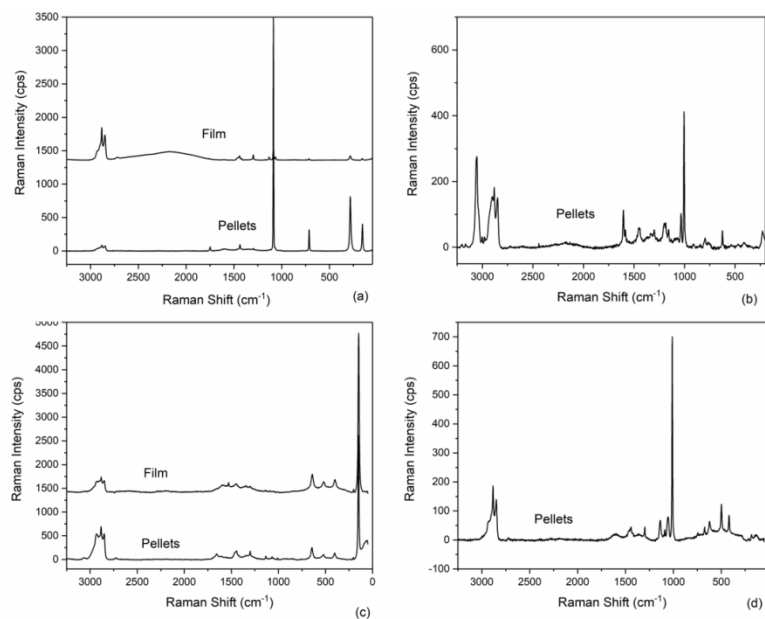
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406 Fig. 2 (printed in colors)

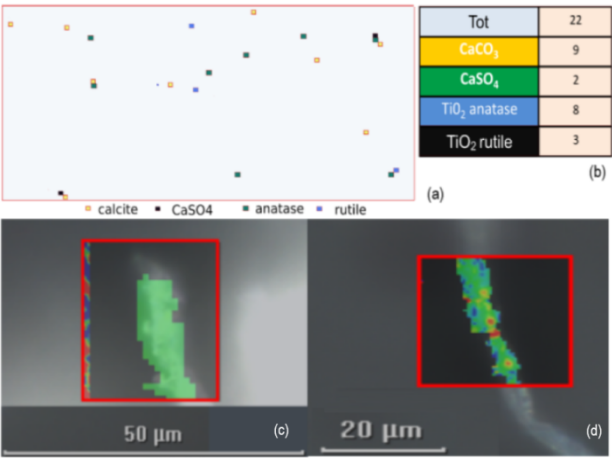


414 Fig. 3



420 Fig. 4 (printed in colors)

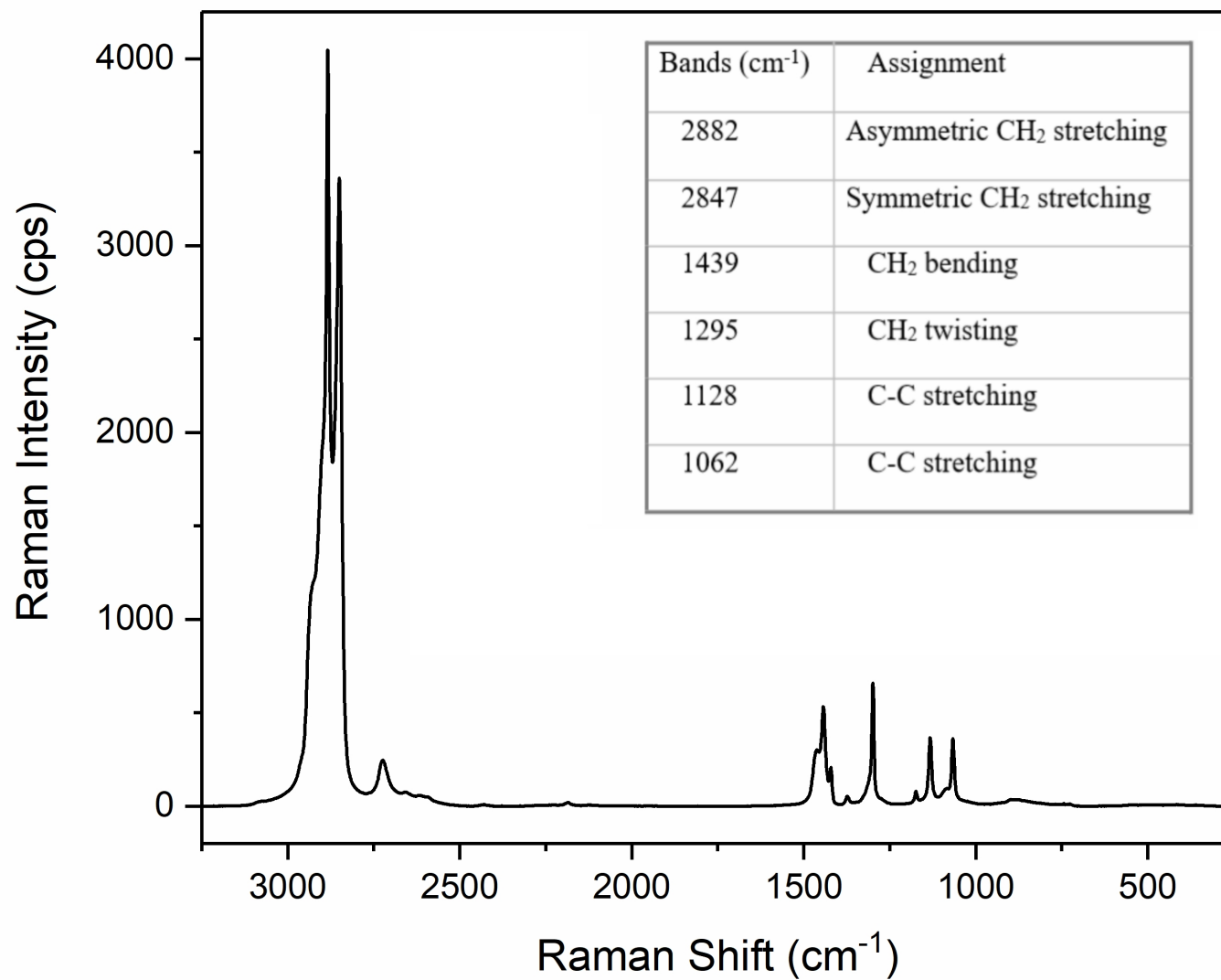
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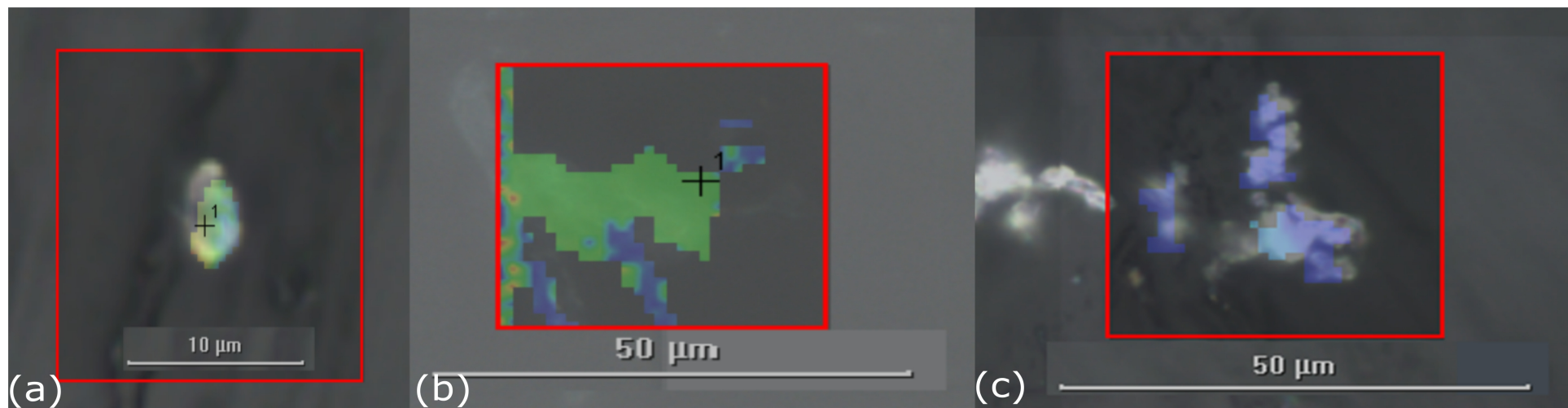


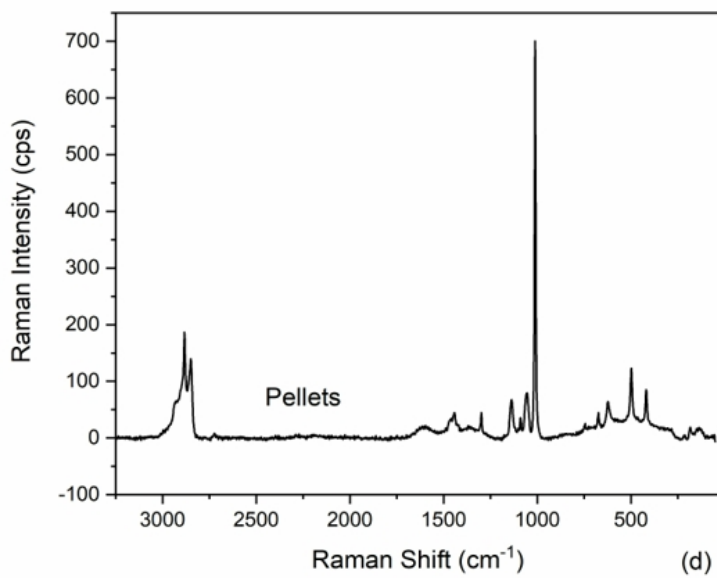
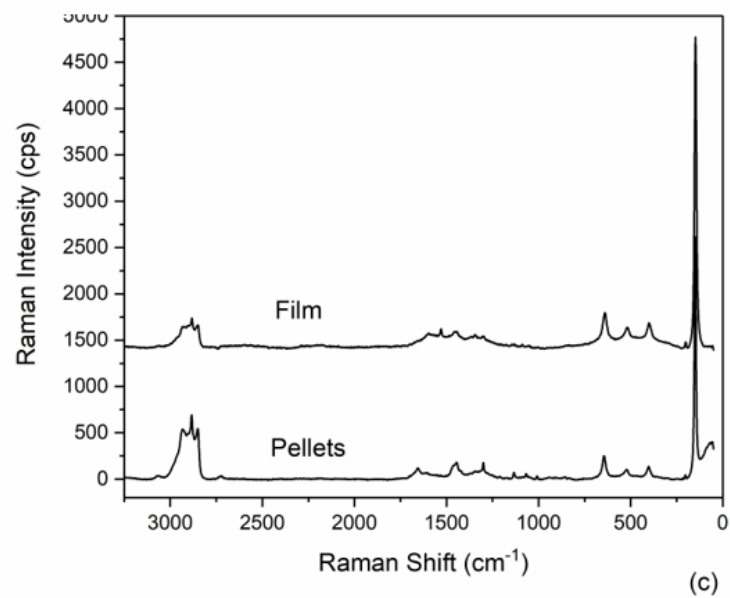
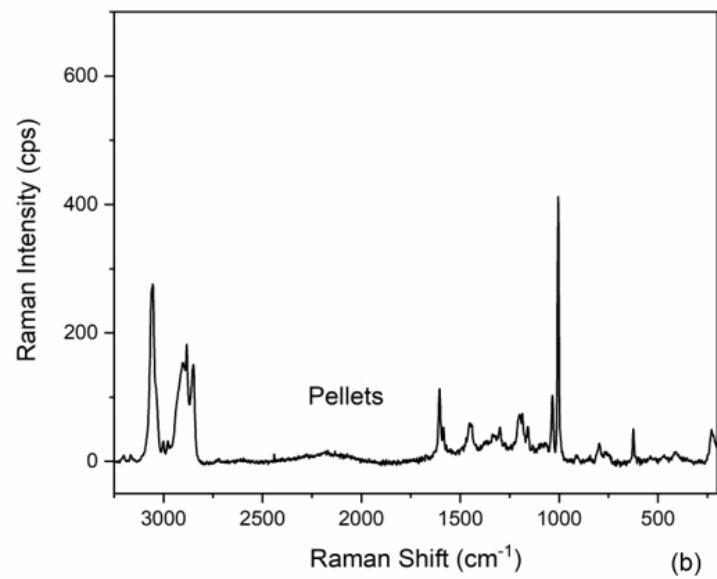
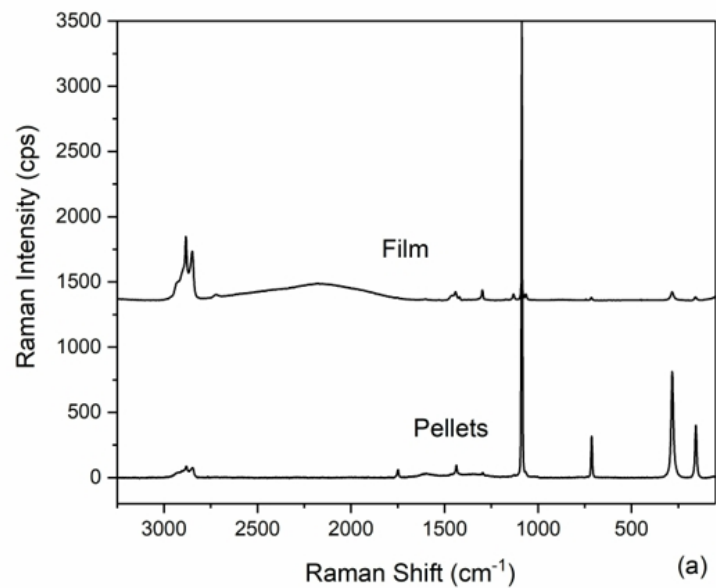
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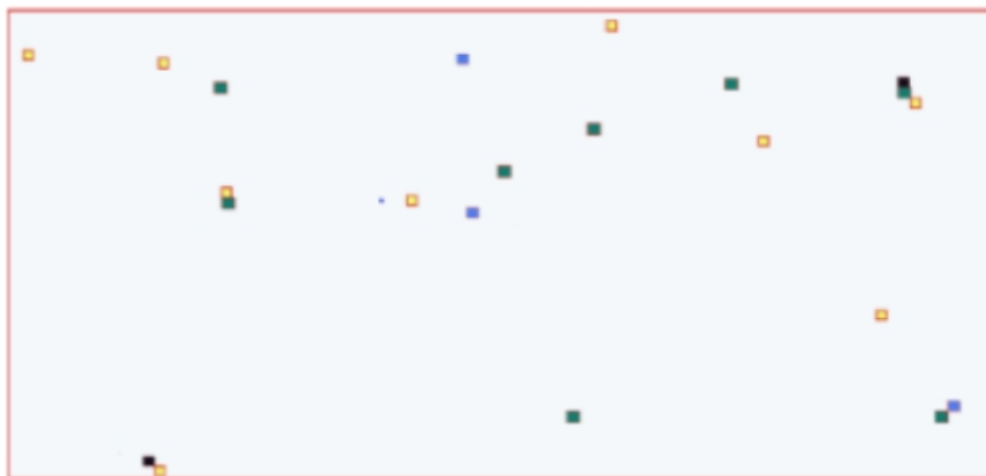
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■ calcite
 ■ CaSO₄
 ■ anatase
 ■ rutile

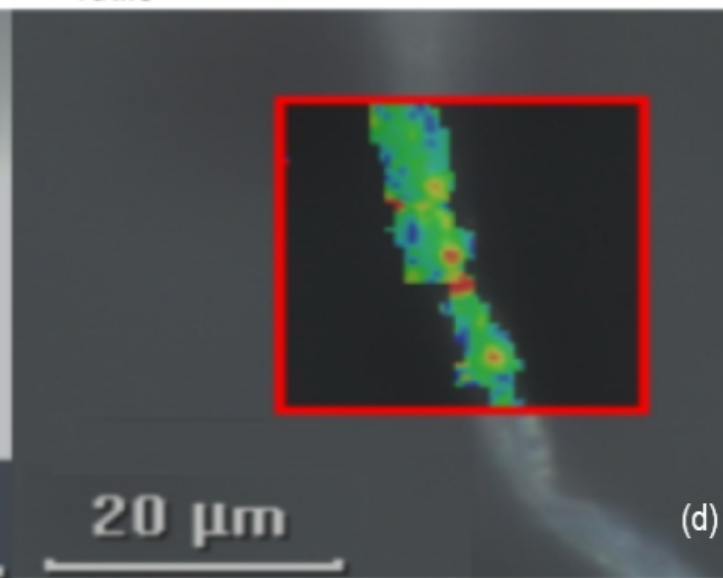
Tot	22
CaCO ₃	9
CaSO ₄	2
TiO ₂ anatase	8
TiO ₂ rutile	3

(b)

(a)



(c)



(d)